REMARKS

Claims 154-167 are pending in the application. All claims have been rejected.

Claims 154-167 have been rejected under 35 USC 103(a) as being obvious over Garger et al. in view of GenBank Accession No. P04062, Boller et al. and Frijters et al. Claims 154-167 have been provisionally rejected under 35 USC 101 for non-statutory double patenting as claiming the same invention as claims of co-pending US Application 11/790991. Claim 158 has been rejected under 35 USC 112, 2nd paragraph, for lacking clarity. Claims 155, 158 and 162 have now been amended. Claims 155-157 have now been canceled, without prejudice. New claims 168- 173 have been added.

35 USC 112, 2nd Paragraph Rejections

The Examiner has rejected claim 158 under 35 U.S.C. 112, 2nd paragraph, as failing to point out and distinctly claim the subject matter of the invention. Applicant acknowledges the technical error in omitting the noun "having" before the terms "an increased uptake...". Claim 158 has now been amended to recite "...having an increased uptake...and catalytic activity", thus further limiting the glucocerebrosidase as claimed in claim 154, and overcoming the rejection on the basis of 35 USC 112 2nd paragraph.

Claim Amendments

Claim 154 has now been amended to include the limitations of the human glucocerebrosidase protein consisting of an amino acid sequence consisting of SEQ ID NO: 8 linked at its C terminal to SEQ ID NO: 2.

Claim 158 has now been amended to recite "...having an increased affinity for...". Claim 162 has been amended to depend from claim 158.

New claims 168-173 have been added. New claim 168 reads on the human glucocerebrosidase protein comprising the amino acid sequence of SEQ ID NO: 8, linked at it's C-terminal to the vacuolar targeting signal peptide as set forth in SEQ ID NO: 2, and wherein the linkage is further defined by the respective amino acid coordinates of SEQ ID NO: 14.

New claim 169 reads on a human glucocerebrosidase protein consisting of an amino acid sequence encoded by a polynucleotide consisting of a first nucleic acid sequence encoding SEQ ID NO: 8 contiguously linked to and in the same reading

frame as a second nucleic acid sequence encoding the vacuolar targeting signal as set forth in SEQ ID NO: 2.

New claims 170 and 171 read on pharmaceutical compositions comprising the human glucocerebrosidase proteins of claims 168 and 169, respectively. New claims 172 and 173 read on cells producing the human glucocerebrosidase proteins of claims 168 and 169, respectively.

Support for such amendments is found throughout the instant specification, for example, page 14, lines 1-5, and Examples 3 and 5 of the instant specification.

Specifically, page 14, lines 1-4 recite:

> "Preferably, the recombinant polynucleotide comprises a first nucleic acid sequence encoding the protein of interest that is in operable link with a second nucleic acid sequence encoding a vacuolar targeting signal peptide derived from the basic tobacco chitinase A gene, which vacuolar signal peptide has the amino acid sequence as denoted by SEQ ID NO: 2..."

Page 23, bridging page 24, recites:

> "The expression vectors or recombinant nucleic acid molecules used for transfecting or transforming the host cells of the invention may be further modified according to methods known to those skilled in the art to add, remove, or otherwise modify peptide signal sequences to alter signal peptide cleavage or to increase or change the targeting of the expressed lysosomal enzyme through the plant endomembrane system. For example, but not by way of limitation, the expression construct can be specifically engineered to target the lysosomal enzyme for secretion, or vacuolar localization, or retention in the endoplasmic reticulum (ER).
>
> In one embodiment, the expression vector or recombinant nucleic acid molecule, can be engineered to incorporate a nucleotide sequence that encodes a signal targeting the lysosomal enzyme to the plant vacuole. For example, and not by way of limitation, the recombinant nucleic acid molecule comprised within the host cell of the invention, comprises a first nucleic acid sequence encoding a lysosomal enzyme that is in operable linkage with a second nucleic acid sequence encoding a vacuolar targeting signal peptide derived from the basic tobacco chitinase A gene. This vacuolar signal peptide has the amino acid sequence as denoted by SEQ ID NO: 2."(page 23, line 26-page 24, line 10).

And

> The term "operably linked" is used herein for indicating that a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Optionally and preferably, operably linked DNA sequences are contiguous (e.g. physically linked) and, where necessary to join two protein-coding regions, in the same reading frame. Thus, a DNA sequence and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s)."(page 24, line 16-25)

In new claim 168, the junction between the C-terminus of the amino acid sequence of the glucocerebrosidase (SEQ ID NO: 8) and the vacuolar targeting signal peptide (SEQ ID NO: 2) is defined by the amino acid coordinates of SEQ ID NO: 14 (see coordinates 519-520). Support for such a limitation is found throughout the instant specification, for example, in SEQ ID NO: 14 (vacuolar targeting signal peptide is italicized):

515 520 525
Thr Tyr Leu Trp His Arg Gln *Asp Leu Leu Val Asp Thr Met*
↑
C-terminal of SEQ ID NO: 8

Other amendments are clerical in nature. No new subject-matter has been introduced.

35 U.S.C. § 103 Rejections: Garger (US Patent Application No:09/993059), in view of Boller (US 6054637) and Frijters (NL-1012782).

The Examiner has rejected claims 154-167 under 35 USC 103(a) as allegedly being unpatentable over Garger et al. ("Garger"), in view of GenBank Accession No. P04062, and Boller et al. ("Boller") and Frijters et al. ("Frijters"). The Examiner's rejections are respectfully traversed. Claims 154, 158 and 162 have been amended. Claims 155-157 have now been canceled.

Claim 154, and all the claims directly or indirectly dependent therefrom, requires a human glucocerebrosidase ("GCD") protein that consists of the amino acid sequence of SEQ ID NO: 8 linked at its C-terminus to the vacuolar targeting signal

peptide as set forth in SEQ. ID. NO.: 2, is glycosylated and comprises at least one exposed mannose, at least one fucose having an alpha (1-3) glycosidic bond and at least one xylose.

The Examiner has alleged that Garger teaches recombinant production of human glucocerebrosidase identical to SEQ ID NO: 8 in transgenic tobacco plants, that Boller teach the advantages of the use of signal peptides for vacuolar sorting of proteins, including SEQ ID NO: 2, and that Frijters teaches an ER signal peptide sequence identical to SEQ ID NO: 1, and that one of ordinary skill in the art would have found it obvious to make a fusion protein comprising the human glucocerebrosidase of Garger, with the vacuolar signal protein of Boller and the endoplasmic reticulum signal sequence as disclosed by Frijters. Applicant disagrees.

Boller teaches away from the vacuolar signal protein of SEQ ID NO: 2

The human glucocerebrosidase protein of claim 154, and claims dependent therefrom, includes the structural limitation of a vacuolar targeting signal protein as set forth in SEQ ID NO: 2 (Asp Leu Leu Val Asp Thr Met). As noted by the Examiner, Boller teaches many variants of the tobacco chitinase vacuolar targeting signal peptide, but is silent regarding the targeting signal peptide as set forth in SEQ ID NO: 2.

SEQ ID NO: 2 differs from the tobacco chitinase sequences taught by the group of Boller (see Neuhaus et al. 1991 Proc. Nat. Acad. Sci. USA 88:10362-10366) in that it is only 7 amino acids in length and has, instead of a Glycine, a single Aspartic acid residue at it's N-terminus (before the Leu-Leu-Val-Asp-Thr-Met sequence), while all the vacuolar targeting sequences taught in Boller include at least one Glycine residue located before the Leu-Leu-Val-Asp-Thr-Met sequence (see, for example, SEQ ID NO: 29 of Boller et al.). Yet further, Neuhaus and Rogers (Neuhaus is a co-inventor in Boller et al.), determined that all the 7 amino acids (with the possible exception of the terminal methionine) of the native tobacco chitinase A signal peptide are both necessary and sufficient for vacuolar targeting (Neuhaus and Rogers, 1998 Plant Mol. Biol. 38:127-144, page 135, right column, 2nd paragraph, lines 1-6). Further investigation into the function of the tobacco chitinase vacuolar targeting signal peptide by Boller's group indicated that deletions and substitutions within the native chitinase vacuolar signaling peptide sequence were detrimental to vacuolar targeting function (with the exception of the terminal Methionine) (Neuhaus

et al, Plant J. 1994;5:45-54, provided herewith). Thus, contrary to the Examiner's contention, Applicant submits that Boller et al., in view of the state of the art at the time of the invention (including Boller's own publications), does not teach adding any vacuolar signal peptide sequence to the C-terminus of any desired protein, and specifically teaches away from vacuolar targeting with the signal peptide as set forth in SEQ ID NO: 2.

Yet further, Applicant submits that, in view of Boller et al.'s clear warning of the detrimental effects of deletions and substitutions within the native chitinase vacuolar signaling peptide sequence, the successful targeting of human glucocerebrosidase to the vacuole with the vacuolar targeting sequence SEQ ID NO: 2, to yield a catalytically active and therapeutically competent enzyme was not only unexpected but also unpredictable.

Garger teaches exclusively secretion of the plant-expressed lysosomal enzymes into the interstitial fluid of whole plants, and teaches away from vacuolar targeting.

While acknowledging that Garger teaches secretion of the recombinant protein to the apoplastic compartment, IF and cell wall matrix, the Examiner has alleged that "nothing in the teachings of Garger et al., or prior art, indicates that directing the human glucocerebrosidase to the vacuole would have a negative effect on the protein" (instant Office Action, page 6-8, paragraph 9). Applicant disagrees.

The Examiner reasons that "a human protein would not have a vacuole targeting signal", thus Garger et al. cannot teach deletion of a vacuole targeting signal in human glucocerebrosidase. But Garger et al. specifically cautions against native lysosomal amino acid sequences acting as endogenous "Carboxy Terminal ProPeptides" (CTPPs), a term used to designate plant targeting and sorting signals(see, for example, Neuhaus and Rogers, Plant Mol Biol 1998). Garger et al. identifies these CTPPs as a problem:

> "Some vacuolar proteins (osmotin, thaumatin, chitinase-I, glucanase-I and a barley lectin), contain sorting information in a CTPP of 7 to 22 AA in length. For several of these proteins secreted isoforms are synthesized without a CTPP domain. In other cases, experimental deletion of the CTPP results in secretion of the recombinant protein to the IF (45-48)....*We hypothesize that a redundant sorting signal may exist in this carboxy-domain that also serves to reduce enzymatic activity in the ER lumen, golgi and trans-golgi*

> *network. This signal appears to function in plant cells, presumably for vacuolar localization.*"(Garger et al, [0106]).

Thus, Garger et al. clearly teaches the existence of CTPPs, or vacuolar sorting signals in the C-terminal sequences of human lysosomal proteins (see also Garger et al., Fig 5 and [0051]), and relates to such sorting signals as an obstacle to be overcome for successful recombinant expression of the human lysosomal proteins in plant cells:

> "In several initial experiments, plant leaves transfected with all constructs accumulated 1-2% of the total soluble plant protein as cross reacting immunologic material (CRIM) using antisera specific for Gal-A in quantitative Western analyses (data not shown). However, enzyme activity was much lower than expected for this amount of CRIM... and furthermore was only 2-4 fold higher than activity due to endogenous plant α-galactosidase isozymes....We reasoned that...the majority of the enzyme was inactivated later in the secretory pathway. This could most likely occur by aggregation in the trans-golgi network...and/or in the plant leaf vacuole."(Garger et al., [0276])

The solution to the potential, undesired vacuolar sorting of the recombinant human lysosomal proteins in plants, according to Garger et al., is deletion (truncation) of these CTPPs (Garger, [0277]), which is reported to have resulted in increased recovery from the interstitial fluid of C-terminal truncated α-galactosidase from tobacco leaves:

> "For several plant proteins vacuolar sorting information is located in a carboxy-terminal propeptide (CTPP; 37,38). During the original cloning and characterization of human Gal-A, Quinn et al., postulated a cathepsin-like potential CTPP cleavage for this enzyme at or near two arginine residues, 26 and 28 AA from the termination codon (39,40). The precise AA sequence at the carboxy terminus has, to our knowledge, never been reported. Because secretion in the plant leaf is through a default pathway **we reasoned that deletion of specific sorting information from a postulated CTPP might yield more active enzyme in the IF**. Analysis of a second set of constructs containing either 12 or 25 AA truncations, with and without the ER retention signal provided dramatic evidence for the significance of this region (See Table 9)."(Garger, et al., [0277], also see Garger et al., Tables 8, 9 and 11).

In reasoning the rejection on the basis of Garger et al. in view of Boller and Frijters, the Examiner has stated that: 1) There are no vacuole targeting signal peptides in a human protein to delete; and 2) Simply because Garger et al. does not teach redirecting the protein of interest to the vacuole is not sufficient for one of skill in the art to reasonably conclude that Garger et al. teaches away from redirecting the protein of interest to the vacuole. In response, Applicant submits that 1) Garger et al. clearly refers to, and indicates the existence of, vacuolar sorting signals (CTPPs) in human lysosomal proteins, and 2) Garger et al. not only does not teach targeting of their recombinant lysosomal enzymes to the plant vacuole, but goes to great lengths (such as modifying–truncating-the lysosomal protein coding sequence) to eliminate any possibility of interference with secretion to the IF. Thus, Applicant submits that Garger specifically teaches away from use of "redundant sorting signals" in the native lysosomal polypeptide (Garger, [0106]) and provides strong incentive, and guidance, for targeting the recombinant lysosomal protein exclusively to secretion to the IF, and eliminating vacuolar targeting.

The Ordinarily Skilled Artisan would not combine Boller with Garger to produce the claimed glucocerebrosidase.

For the reasons cited above, one of ordinary skill in the art with Garger in hand, would not be motivated to combine the secreted lysosomal protein as taught in Garger with the vacuolar targeting signal for use with naturally occurring plant proteins as taught by Boller for production of the claimed human recombinant glucocerebrosidase protein (and plant cells and pharmaceutical compositions comprising said protein).

First, Boller does not teach the expression of any mammalian or human polypeptides in plant cells, and certainly not GCD. Yet further, the invention as claimed requires the vacuolar targeting signal peptide "as set forth in SEQ ID NO: 2". Boller not only fails to teach SEQ ID NO: 2 as a vacuolar targeting signal peptide, but Boller, in view of the state of the art, teaches away from using the vacuolar targeting signal peptide of SEQ ID NO: 2.

Second, in view of the express teaching in Garger to not use, or even remove a vacuolar targeting sequence, the ordinarily skilled artisan would not combine Garger with Boller, which is cited for teaching a vacuolar-targeting peptide.

The deficiencies of Garger and Boller are not remedied by Frijters. Frijters is silent regarding lysosomal enzymes, and does not teach or motivate vacuolar targeting of plant expressed enzymes. Frijters merely reports the sequence of an ER targeting signal as set forth in SEQ ID NO: 1 of the instant specification.

Yet further, the inventors surprisingly and unexpectedly found that the recombinant human glucocerebrosidase is glycosylated to produce a terminal mannose (in a major fraction thereof), retains SEQ ID NO: 2 post transit without impairing enzyme activity, as tested by both enzymatic assay and by macrophage uptake. As shown in the examples section the polypeptide of the present invention (page 42) retains the signal peptide as is evidenced by mass-spectrometry.

Applicant submits that Garger cannot be reasonably combined with Boller, with or without Frijters to produce the claimed human glucocerebrosidase protein. Withdrawal of the rejections based on obviousness is respectfully requested.

While strongly traversing the Examiner's rejection of the claims on the basis of the combination of Garger, Boller and Frijters, as detailed herein, and solely in order to expedite prosecution in this case, Applicant has amended claim 154 to include the limitation of the amino acid sequence of the human glucocerebrosidase protein consisting of SEQ ID NO: 8 linked at its C terminus to the vacuolar signal peptide as set forth in SEQ ID NO: 2. Applicant believes that such an amendment now further distinguishes the claimed human glucocerebrosidase protein, compositions and cells expressing the same, from any polypeptides taught, suggested or inferred by any of Garger, Boller or Frijters, alone or in combination.

Double Patenting

The Examiner has rejected claims 154-167 on the grounds of provisional double patenting as claiming the same invention as claims 23-25 of co-pending US Patent Application No. 11/790,991. Claims 155-157 have now been canceled, rendering moot the Examiner's rejections thereof.

Issues of provisional and obviousness-type double-patenting and the submission of a terminal disclaimer will be further considered with respect to US Patent Application No. 11/790,991 upon indication by the Examiner of allowable claims in the present case.

In view of the foregoing amendments and remarks, claims 154 and 158-173 are deemed to be allowable. Their favorable reconsideration and allowance is respectfully requested.

Respectfully submitted,

/Jason H. Rosenblum/

Jason H. Rosenblum
Registration No. 56437
Telephone: 718.246.8482

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Enclosures:
- Request for Continued Examination (RCE)
- Petition for Extension (3 Months)
- Declaration of Inventor Yoseph SHAALTIEL
- CV of Inventor Yoseph SHAALTIEL
- 10 References:
    a) Cramer et al 1999;
    b) Aviezer et al, PLoS 2009;
    c) SciAmericanblog 2009;
    d) Gaucher's Assoc UK website 2010;
    e) Press releases I;
    f) Press releases II;
    g) Press releases III;
    h) Neuhaus et al, Plant J. 1994;5:45-54;
    i) Neuhaus et al. 1991 Proc. Nat. Acad. Sci. USA 88:10362-10366;
    j) Neuhaus and Rogers, 1998 Plant Mol. Biol. 38:127-144